Determination of sensitivity of male Wistar rats to an equal dose of ketamine/xylazine injection at anesthetic dose in a chronic model of hypernatremia in comparison with control group

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ABSTRACT

Objective: To determine the sensitivity to an equal dose of ketamine/xylazine injection at anesthetic dose in a chronic model of hypernatremia.

Methods: This study was conducted at the Department of Physiology, Zanjan University of Medical Science, Zanjan, Iran in 2004. Sixty male Wistar rats, weighing 250±20 g were randomly allocated to 3 groups. The control group was provided with tap water, and first and second test groups consumed 1% and 2% salt concentrations for 144 hours. One hundred mg/kg ketamine and 10 mg/kg xylazine were used as an anesthetic agent. The measured anesthetic parameters comprises of righting reflex latency, required time for establishment of animal's immobility, immobility period, required time for appearance of animal's mobility and complete re-establishment of the righting reflex.

Results: The required time for inhibition of the righting reflex and animal's mobility in the second group was significantly shorter than the first and control groups. Immobility period, required time for appearance of animal's mobility and complete re-establishment of the righting reflex in the second group were significantly longer than the first and control groups.

Conclusion: Hypernatremia increases the speed of transition from different steps of ketamine/xylazine anesthesia with significant delay in immobility period and recovery from anesthesia in rats, hence, anesthetic dose reduction in hypernatremia is necessary.

Saudi Med J 2007; Vol. 28 (10): 1485-1488

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Received 15th January 2007. Accepted 7th May 2007.

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Disorders of body fluids are among the most commonly encountered problems in the practice of clinical medicine.[1] Disorder of water imbalance manifests as hyponatremia and hypernatremia.[2] Sodium is regulated within narrow limits (137-141 mmol/L).[3] As with humans, the physiologic serum sodium ranges are 135-146 mmol/L in rats.[4] Sodium imbalances are commonly encountered in clinical practice and can have a substantial impact on the prognosis of the patient.[5] Hypernatremia is an increase in extracellular sodium concentration above 145 mmol/L.[6] Hypernatremia is one of the most common electrolyte disorders with the incidence of 1-3% in non-hospitalized patients, and the prevalence among nursing home patients who require acute hospitalization has been reported to be more than 30%.[7] Hypernatremia is associated with significant morbidity and mortality especially in children.[8] Hypernatremia can result from water loss or sodium retention.[9] Hypernatremia can occur with normal, increased, or decreased total body sodium content.[10] Hypernatremia could be hypovolemic, hypervolemic, or evolemic.[11] The function of internal systems can be influenced significantly during the hypernatremia; even anesthesia may be affected by this electrolyte imbalance. Hypernatremia increases the halothane minimum alveolar concentration (MAC) in dogs by as much as 43%. Hypernatremia increases the MAC of inhalation anesthetic agents, too.[12] Anesthesiologists face conflicts with anesthesic complications during the hypernatremic state.[13] As hypernatremia increases MAC of inhalation anesthetic agents; thus, we believed that hypernatremia decreases sensitivity to anesthetic drugs and increases the anesthetic dosage. The objective of this study is to determine the sensitivity to an equal dose of ketamine/xylazine injection at anesthetic dose in a chronic model of hypernatremia in rats.

Methods. This study was conducted at the Department of Physiology, Faculty of Medicine, Zanjan University of Medical Science, Zanjan, Iran from April 2003 to April 2004. The study was approved by animal research committee of Zanjan University of Medical Science and the principles of laboratory animal care (National Institutes of Health publication No. 86-23, revised 1985) were followed in this study. The animals were provided by the Iranian Razi Institute, and chemical drugs including Ketamine hydrochloride and xylazine hydrochloride of Alfasan Co. (Woerden, Holland) and Taurine and Nacl of Merck Co. (Darmstsdt, Germany) Brands were purchased.

Rats drinking 0.1 M (Molar) Taurine plus 1.8% sodium chloride (NaCl) developed a mean plasma sodium concentration of 160 ± 18 mmol/L by the sixth day, as compared with 137 ± 1.6 mmol/L of the control group consuming potable water.[14] On that base, 0.1 M Taurine plus 1% and 2% salt concentrations were selected for inducing hypernatremia. The 1% and 2% salt concentrations were prepared by adding 10 and 20 g salt separately to one liter of distilled water. The 1% salt concentrations contained 170 mmol Na and 2% salt concentrations contained 340 mmol Cl. The 1% salt concentrations osmolarity is approximately 340 mmol and 2% salt concentrations osmolarity is approximately 680 mmol. Ketamine/xylazine is a commonly used anesthetic drug for laboratory rats. A combination of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine (KX) was selected as anesthetic drug with intraperitoneal route injection.[15] Animals were housed 5 per cage under a standard 12 hours light/dark cycle with free access to food and constant room temperature of 21°C. Prior to salt administration, physical examination was performed and the healthy animals were selected for study. Sixty male Wistar rats, weighing 250 ± 20 g were allocated randomly to 3 groups.

During the experiment, the control group used potable water, and the test group was deprived from drinking water. The first and second test groups consumed 0.1 M Taurine plus 1% and 2% salt concentration as the sole source of potable water for 144 hours. All groups were fed on same diet, containing approximately 0.5% salt, and provided with similar living conditions for all groups. The onset of water deprivation, in the test group, was considered as the beginning of the experiment. Daily examinations and subsequent recording of health status of each animal were performed. Blood samples were drawn to measure the serum sodium level at the onset of the experiment and before anesthesia induction. The serum sodium level was measured by flame photometer, of Cornning 480 Brand (Tokyo, Japan). In order to prevent anesthetic complications, the rats were deprived of eating and drinking an hour prior to anesthesia induction. A combination of 100 mg/kg ketamine hydrochloride and 10 mg/kg xylazine (KX) were prepared, and then calculated dose on weight bases were injected intraperitoneally. Subsequently, righting reflex latency, required time for establishment of animal's immobility, period of immobility, reduced responsiveness to external stimuli, required time for appearance of animal's mobility and complete re-establishment of the righting reflex were recorded. Complete re-establishment of the righting reflex considered as full recovery from anesthesia, the time when animals could spontaneously moved on the table. The number of deceased animals and the time of death was recorded. In dead animals, craniotomy was performed to detect any possible intracranial hemorrhages. Conducting this experiment in test groups accompanied with some mortality, except for, required time for appearance of animal's mobility and full recovery from anesthesia, the rest of data are included in final results. The remaining live animals in the test groups were treated by gradual exposure to tap water.

The data were expressed as mean±SD and different groups analysis of variance test with the help of SPSS Software, version of 11.5, and $p < 0.05$ was considered as significant changes.

Results. In test groups, no animal death was observed during the period of salt administration. At the onset of the experiment there was no significant differences in the mean sodium levels between the groups (**Table 1**). After salt administration, the mean sodium level in the second group was significantly greater than the first and control group (p=0.004) (**Table 1**). The mean serum sodium level of p=0.003 in the second group was significantly greater than the first and control group (p=0.004). This parameter in the first group was significantly greater than the control group with a p value of 0.04.

The righting reflex latency in the second group was significantly shorter than the first group (p=0.03) and in the control group (p=001); this duration in the first group was significantly shorter than the control group (p=0.045). The required time for establishment of animal's immobility in the second group was significantly shorter than the first group (p=0.04) and

Table 1 - Comparison of mean serum sodium levels in different groups at the beginning of experiment and 144 hours after salt concentrations consumption in the test groups

Groups	Serum sodium levels before salt solutions consumption	Serum sodium levels before anesthesia induction
Control	137.1 ± 2.6 mmol /L	138.5 ± 2.4 mmol /L
Test 1	137.9 ± 2.1 mmol /L	147.8 ± 2.6 mmol /L
Test 2	138.4 ± 1.8 mmol /L	152.4 ± 3.3 mmol /L

Table 2 - Comparison of different anesthesia parameters in the experimental groups.

Groups	Mortality Rate (%)	Time of death after injection (Mean ±SD)	Complete re-establishment of the righting reflex (Mean ±SD)	Required time for appearance of animal's mobility (Mean ±SD)	Required time for animal's immobility (Mean ±SD)	Required time for inhibition of the righting reflex (Mean ±SD)
Control	0	---	245 ± 25 min	212 ± 17 min	10 ± 2min	7 ± 2 min
Group 1	15	25 ± 5 min	226 ± 16.5 min	186 ± 20 min	7 ± 1.5min	5 ± 1 min
Group 2	40	17 ± 3 min	195 ± 11.5 min	155 ± 15 min	5 ± 1.5 min	3 ± 1 min

in the control group (p=0.002); this duration in the first group in comparison with the control group was a borderline significant of p=0.05. In contrast, the period of drug efficacy (KX) and animal's immobility increased in test groups significantly. The required time for appearance of animal's mobility with a p value of 0.002 in the second group was significantly longer than the first group and p=0.005 in the control group. This duration in the first group was also longer than the control group with a p value of 0.03. Complete re-establishment of the righting reflex (full recovery) with a p value of 0.025 in the second group was significantly longer than the first group and p=0.006 in the control group. This duration in the first group was significantly longer than the control group with a p value of 0.015. **Table 2** shows the comparison of different anesthetic parameters between experimental groups. Following craniotomy, different forms of intracranial hemorrhages were observed in most of the hypernatremic rats.

Discussion. This study presented several key findings in relation to the sensitivity of male Wistar rats to an equal dose of ketamine/xylazine injection at anesthetic dose in a chronic model of hypernatremia in comparison with the control group. The findings are as follows: First, serum sodium level in the second group is higher than other groups and righting reflex latency and required time for establishment of animal's immobility in the second group was shorter than the first and control group. Second, the period of immobility, required time for appearance of animal's mobility and full recovery in the second group was longer than the first and control group. Third, the speed of transition from different stages of anesthesia in the second group was faster than the first and control group. Forth, sensitivity to an equal dose of ketamine/xylazine injection at anesthetic dose in the second group was higher than the first and control group. Fifth, negligence of dose reduction during anesthesia is associated with severe complications and mortality in the test groups.

The form of hypernatremia in this study is of the hypovolemic type. Hypovolemia reduces the distributable volume of intravenous drugs, which necessitates dose reduction.[16] Ketamine/xylazine dosage was not reduced in the test groups, hence, the volume of ketamine/xylazine distribution was decreased, which led to increase concentrations in the test groups, therefore, ketamine/xylazine's efficacy in the test groups appeared faster than control group. Acute manipulation of daily sodium intake dose alters renal function and hepatic CYP (Cytochrome P450) isoforms, this should be taken into consideration when using these rat models.[17] Hypernatremia lead to cellular damage in hepatocytes.[18] It also leads to hyperlipemia and a fatty liver.[19] Due to hepatic and kidney tissue changes, ketamine/xylazine's metabolic processes may become longer in the test groups in comparison with the control group. Reduction in the distributed volume of ketamine/xylazine's leads to increased anesthetic drug concentration in body fluids. Aside from that, slower ketamine/xylazine's metabolism, increased the duration of ketamine/xylazine's efficacy in the test groups. Hypernatremia solely can be life-threatening.[6] It primarily affects the central nervous system[18] and leads to a water flow across the blood-brain barrier from brain to plasma, and brain volume decreases, resulting in intracerebral hemorrhage.[20]

Hypernatremia increases sensitivity to ketamine/xylazine anesthesia in rats; as a result, dose reduction should be taken into consideration. Negligence of dose reduction will lead to severe complications and mortality rates in hypernatremic rats. Ketamine is considered to be a potent cerebral vasodilator; hence, its administrations are avoided in patients with known intracranial pathologic disorders.[21] Certain death causes in the test group were due to ketamine impacts. The obtained results in this study coincides with similar findings released by other researchers. During anesthesia, the anesthesiologist will face lot of complications in the hypernatremic state.[13] The prevalence of hypernatremia among patients who required immediate hospitalization is high, and needs prompt surgical intervention, hence with attention to this fact and limited numbers of studies performed on the effect of hypernatremia on anesthesia, future comprehensive and applied studies are strongly recommended in this field.

In conclusion, hypernatremia increases the speed of transition from different steps of ketamine/xylazine anesthesia with significant delay in the immobility period and recovery from anesthesia in rats, hence, anesthetic dose reduction in hypernatremia is necessary.

Acknowledgments. *This work was supported by Zanjan University of Medical Sciences. The result of this research was presented as a poster in the 11th World Congress on Anesthesiology and some other congress.*

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